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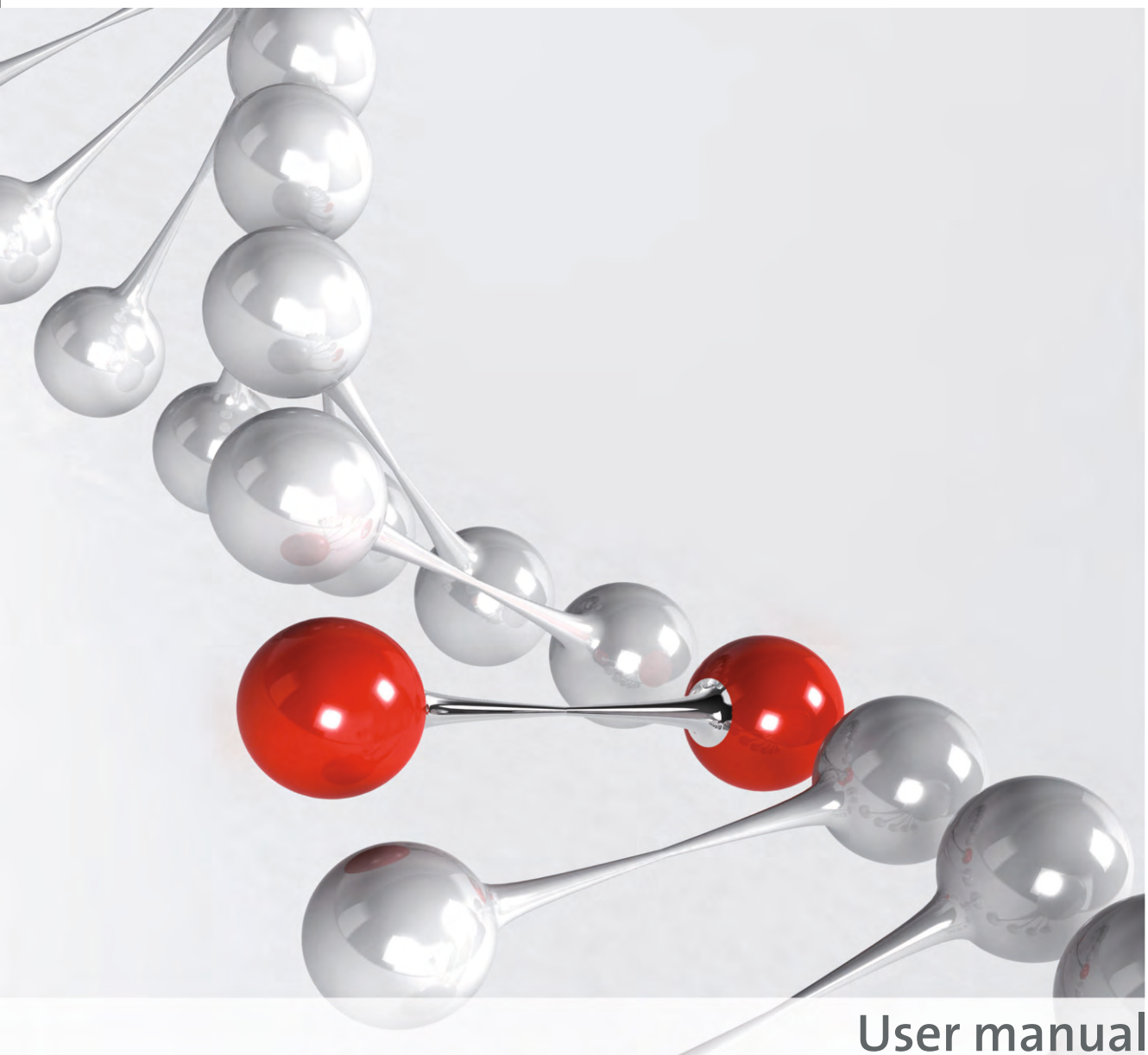

User manual
InviMag® Universal Bacteria Kit/ KF96

for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher

for automated purification of bacterial DNA from different types of specimen: swabs, tissue, food, paraffin embedded tissue, urine, blood or water samples with magnetic beads

REF 7433300x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Universal Bacteria Kit /KF96

The **InviMag® Universal Bacteria Kit/ KF96** is the ideal tool for isolation and purification of bacterial DNA with enhanced performance and reproducibility from different clinical specimen like whole blood, swabs, biopsy material and from cell-free body fluids like urine, serum, plasma and synovial fluids in a convenient 96 well format using the innovative STRATEC Molecular technology in combination with Thermo Fisher instruments. The use of KingFisher instruments allow time-saving processing of large sample numbers without any cross-contaminations. Furthermore, the user is flexible in the choice of the starting amount material.

The kit composition of the **InviMag® Universal Bacteria Kit/ KF96** is designed for the use on **KF96** or **KFflex96** instruments**.**

The kit is neither validated for the isolation of human or bacterial genomic DNA from fecal samples nor for viral nucleic acids or total RNA isolation.

Trademarks: InviMag®, Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of InviMag® Universal Bacteria Kit/ KF96

Store the **MAP Solution A** at 4°C! Store dissolved **Proteinase K** at -20°C!
Store all other kit components at room temperature (RT)!

	1 x 96 extractions	**5 x 96 extractions**
Catalogue No.	7433300100	7433300200
Lysozyme Buffer	15 ml	50 ml
Lysozyme	150 mg	500 mg
Lysis Buffer HL	30 ml	110 ml
Proteinase K	2 x 1.1 ml working solution	10 ml working solution
Binding Buffer HL	8 ml final volume 32 ml	30 ml final volume 120 ml
MAP Solution A	2 x 1.1 ml	10.5 ml
Wash Buffer I	80 ml final volume 160 ml	2 x 125 ml final volume 2 x 250 ml
Wash Buffer II	60 ml final volume 200 ml	4 x 60 ml final volume 4 x 200 ml
Elution Buffer	30 ml	110 ml
2.0 ml Deep Well Plate	5	5 x 5
KF96 Tip Comb for DW magnets	1	5
200 µl Elution Plate	2	2 x 5
1.5 ml Receiver Tubes	2 x 50	10 x 50
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 24 ml of 99.7% isopropanol to the **Binding Buffer HL.** Mix by intensive shaking. Always mix shortly before use. Dilute each **Proteinase K** tube by addition of 1.1 ml ddH_2O, mix thoroughly until completely dissolving and store at -20°C! Dilute all **Lysozyme** directly before use with all of the provided **Lysozyme Buffer.** Store diluted Lysozyme at -20°C Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed Add 140 ml of 96-100 % ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed	Add 90 ml of 99.7% isopropanol to the **Binding Buffer HL.** Mix by intensive shaking. Always mix shortly before use. Dilute **Proteinase K** by addition of 10 ml of ddH_2O, mix thoroughly until completely dissolving and store at -20°C! Dilute all **Lysozyme** directly before use with all of the provided **Lysozyme Buffer.** Store diluted Lysozyme at -20°C. Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed

Kit contents of InviMag® Universal Bacteria Kit /KF96 w/o plastic

Store the **MAP Solution A** at 4°C! Store dissolved **Proteinase K** at –20°C!
Store all other kit components at room temperature (RT)!

	1 x 96 extractions	5 x 96 extractions
Catalogue No.	7433300150	7433300250
Lysozyme Buffer	15 ml	50 ml
Lysozyme	150 mg	500 mg
Lysis Buffer HL	30 ml	110 ml
Proteinase K	2 x 1.1 ml working solution	10 ml
Binding Buffer HL	8 ml final volume 32 ml	30 ml final volume 120 ml
MAP Solution A	2 x 1.1 ml	10.5 ml
Wash Buffer I	80 ml final volume 160 ml	2 x 125 ml final volume 2 x 250 ml
Wash Buffer II	60 ml final volume 200 ml	4 x 60 ml final volume 4 x 200 ml
Elution Buffer	30 ml	110 ml
1.5 ml Receiver Tubes	2 x 50	10 x 50
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 24 ml of 99.7% isopropanol to the **Binding Buffer HL.** Mix by intensive shaking. Always mix shortly before use. Dilute each **Proteinase K** tube by addition of 1.1 ml ddH_2O, mix thoroughly until completely dissolving and store at -20°C! Dilute **Lysozyme** directly before use with the provided **Lysozyme Buffer.** Store diluted Lysozyme at -20°C Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed Add 140 ml of 96-100 % ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed	Add 90 ml of 99.7% isopropanol to the **Binding Buffer HL.** Mix by intensive shaking. Always mix shortly before use. Dilute **Proteinase K** by addition of 10 ml of ddH_2O, mix thoroughly until completely dissolving and store at -20°C! Dilute **Lysozyme** directly before use with the provided **Lysozyme Buffer.** Store diluted Lysozyme at -20°C. Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well Plate	5	25
KF96 Tip Comb for DW magnets	1	5
200 µl Elution Plate KF96	2	10

Symbols

Manufacturer

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of **the InviMag® Universal Bacteria Kit/ KF96**, except **MAP Solution A**, are stable for at least 12 months. **MAP Solution A** is stable for at least 6 months.

All buffers and kit contents of the **InviMag® Universal Bacteria Kit/ KF96**, except **Lysozyme, dissolved Proteinase K** and **MAP Solution A,** should be stored at room temperature.

Proteinase K: Dissolved Proteinase K should be stored in aliquots at –20°C. Avoid multiple freezing and thawing cycles.

Lysozyme: Lyophilized Lysozyme should be stored at 4-8°C. Dissolved Lysozyme should be stored in aliquots at –20°C. Avoid multiple freezing and thawing cycles.

MAP Solution A: The beads must be stored at 2-8°C.

Wash Buffers: Wash Buffers charged with either ethanol or isopropanol should be stored at room temperature and must be appropriately sealed. If any precipitates within the provided solutions are visible, redissolve them by carefully warming up to 30°C.

Room temperature is defined as range from 15–30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Universal Bacteria Kit/ KF96** for applications as described in the manual. Purchasers must determine the suitability of the product for its particular use. Should any product fail to perform in applications as described in the manual, STRATEC Molecular will check the lot and if a problem in the lot is detected, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Universal Bacteria Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If any questions or problems arise regarding any aspects of **InviMag® Universal Bacteria Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany	**+49-(0)30-9489-2901/ 2910**
from abroad	**+49-(0)30-9489-2907**

or contact your local distributor

Intended use

The **InviMag® Universal Bacteria Kit /KF96** allows rapid and economic isolation of high quality bacterial DNA from up to 100 µl of fresh or frozen blood, plasma, serum, cell-free body fluids of human origin like urine, as well as rinsed liquid from swabs or cell pellets in a convenient 96 well format using the **InviMag® technology** and the **KF96** or **KFflex96** instrument from Thermo Scientific. Fresh or frozen blood, plasma or serum from common blood collection systems can be used, stabilized with EDTA or citrate, but ***not*** with heparin. The extracted high quality bacterial DNA is suitable for a wide variety of downstream applications.

For reproducible and high yields, appropriate sample storage is essential. Yields may vary from sample to sample depending on factors such as the health of the donor, patient medication or sample storage conditions. The purified DNA is of high quality and free of proteins, nucleases and/or other impurities and is ready-to-use for different downstream applications like PCR, quantitative PCR, real-time PCR or other routine downstream methods.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. The kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of human or bacterial genomic DNA from fecal samples nor for viral nucleic acids or any kind of RNA isolation.

The included chemicals are only useable once.

Differing the starting material or flow trace may lead to inoperability. Therefore, neither a warranty nor guarantee will be given, implied or expressed if the respective protocol is changed.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used, e.g. in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory, after the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastics are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The product with its contents is not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid direct skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Universal Bacteria Kit /KF96** procedures for residual risk materials. Therefore, all liquid waste should be handled and discarded accordingly to local safety regulation.

European Community risk and safety phrases for the components of the **InviMag® Universal Bacteria Kit /KF96** to which they apply, are listed below as follows:

Lysis Buffer HL:

warning
H302-315-319 P280-305-351-338

Proteinase K

danger
H315-319-334-335 P280-305-351-338-310-405

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

H302:	Harmful if swallowed.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. If present, remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P273:	Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**
inside of USA: **1 – 800 – 535 – 5053**

Product characteristic of the InviMag® Universal Bacteria Kit /KF96

Starting material	Yield	Time
up to 10^9 bacteria, 1-10 mg tissue, biopsy, paraffin embedded tissue, 1-100 µl whole blood, 200 µl cell-free body fluid, 15-50 ml urine, paper points, swabs, up to 1 l water	depends on the starting material	approx. 70 minutes (including lysis)

The **InviMag® Universal Bacteria Kit /KF96** is designed for a semi-automated preparation of bacterial DNA form different clinical sample materials in a 96 well format using magnetic beads and the KF96 or KFflex96 instrument from Thermo Scientific.

The isolation process is based on the patented **InviMag®** technology which is used for isolation of DNA by binding of the nucleic acids onto magnetic particles.

In the first step, all samples are adjusted to a final sample volume of 100 µl using 1x PBS or distilled water before the 96 well format is applied.

The samples are pretreated with **Lysozyme** at 37°C to break the bacterial cell wall in presence of lysis buffer. Proteins are degraded during lysis upon addition of **Proteinase K** in a step performed at 56°C on the instrument. The lysis efficiency is improved by mixing the samples during the lysis step controlled by the magnetic rods of the King Fisher instrument. After addition of magnetic beads and binding buffer, to adjust optimal binding conditions, the DNA, bound to the magnetic beads, are transported through several washing steps and a final elution step. The procedure requires only minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contaminations.

The bacterial DNA extraction protocols described herein are designed for walk-away automated preparation of bacterial DNA using different materials in combination with the InviMag® technology. The extracted bacterial DNA is of very high quality and suitable for a wide variety of downstream applications or can be stored at -20°C for subsequent use.

The isolation protocols and all required buffers are optimized to provide highest yields and purities of the extracted bacterial DNAs.

- PCR*
- Real-time PCR
- RFLP analysis
- Restriction enzyme digestion

Yield and quality of bacterial DNA

The amount of isolated, purified DNA depends on the type of sample, bacterial titer, sample source, transport, storage and age. Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.

Yields of bacterial nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

* Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present RNA.

** The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Yield and quality of isolated bacterial DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed accordingly to the manufacturer´s specifications.

Protocol validation

Verification Testing

The bacterial DNA extraction protocol was intensively tested on a KFflex96 instrument with the provided reagents and consumables. Typical results for the extraction of bacterial DNA from buffer, plasma and blood are shown below. Actual results can vary, depending upon sample age, quality, type, bacterial titer and host.

Samples

For testing, frozen cell pellets from the Gram-positive bacterium *Bacillus subtilis* were used in different dilutions in combination with different matrices. The cells were grown in an over-night culture and the derived cell pellets from 1 ml of this culture were stored at -20°C until further use. In all experiments, a fresh pellet was used from the -20°C stock. At all times, thawed and unused cells were discarded after the experiment. The detection was done by an in-house *Bacillus subtilis* real-time PCR based detection assay. All real-time PCRs were performed on a Corbett Rotor-Gene 3000.

PCR Inhibitor and Cross Contamination Test

To maximize the detection of any potential contamination event, positive and no sample controls were arranged in alternating wells (in a “checkerboard” pattern ***Fig. 1***) using the **InviMag® Universal Bacteria Kit /KF96**. Out of those samples, ***Fig. 2*** shows a real-time PCR run of the extracted DNAs. The PCR was done with an in-house SYBR Green based real–time PCR assay on a Corbett Rotor-Gene 3000 machine.

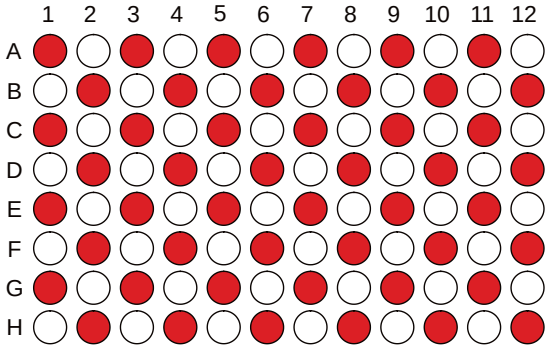


Fig. 1 **‘Checkerboard Pattern:** Utilized for the cross-contamination analysis test. Samples (red) and no sample controls (white) arranged in alternating wells.

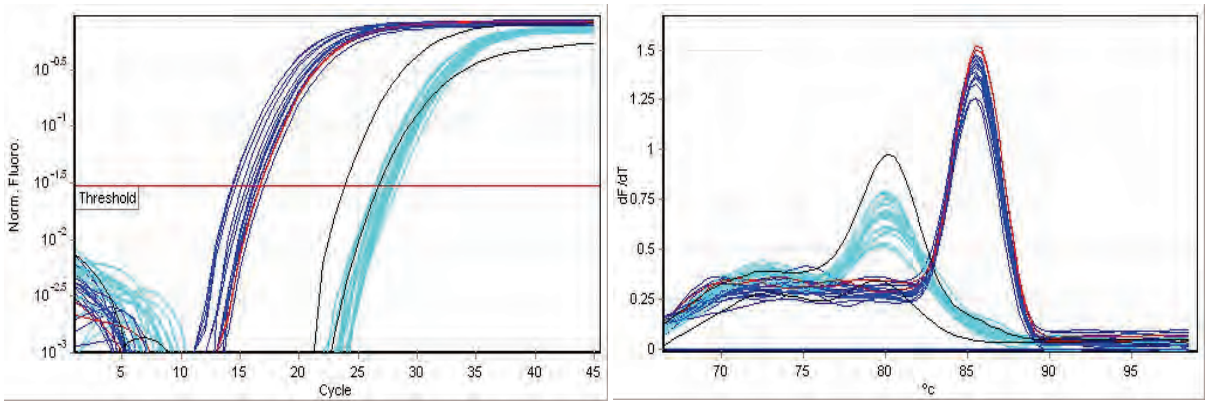


Fig. 2: Real-time PCR results (left figure) from positive samples (host: *Bacillus subtilis*; dark blue) and no sample controls (water; cyan) arranged in a Checkerboard pattern. NTC (no template control; black) and PTC (positive template control; red) are shown also. The right figure shows the corresponding melting curves.

Color	Name	Ct	Rep. Ct	Rep. Ct Std. Dev.
■	Sample	16.19	16.38	2.75
■	No sample	26.54	27.38	0.54
■	PTC	16.69	16.65	0.05
■	NTC	23.82	25.27	2.06

Tab. 1 The table shows the high reproducibility of the mean Ct value and the standard deviation.

Reproducibility

To demonstrate the reproducibility of the **InviMag® Universal Bacteria Kit /KF96** bacterial DNA extractions were performed in dilution series. Typical results are shown below:

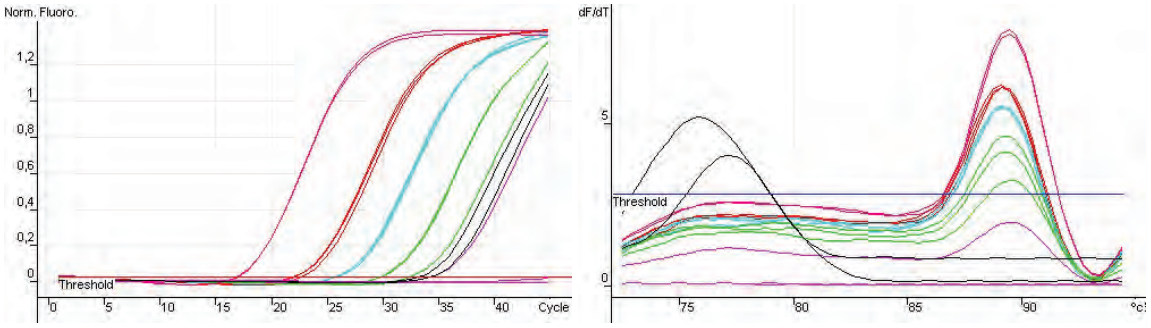


Fig. 3 On the left side real-time PCR results of an extracted DNA from a dilution series of *B. subtilis* in TE buffer is shown. On the right side the corresponding melting curves for the resulting PCR products are shown.

Color	Name	mean Ct	mean Ct Std. Dev.
	10e-3	18.90	0.09
	10e-4	22.45	0.16
	10e-5	26.04	0.27
	10e-6	30.91	1.03
	PTC	14.36	0.08

Tab. 2 Ct values and standard deviations for the real-time PCR are shown above in figure 3. Colors belong to the curves in figure 3.

Principle and procedure

The **InviMag® Universal Bacteria Kit /KF96** procedure comprises the following steps:

- sample pre-treatment and normalization of all samples
- lysis of bacterial cells
- binding of bacterial DNA to magnetic beads
- washing and elimination of ethanol
- elution of total DNA (bacterial, mitochondrial)

The samples are lysed in optimized buffers and enzyme mixtures. The lysates are transferred to the subsequent automatic purification procedure based on magnetic beads. The DNA binds to the magnetic particles, is purified by several washing steps and then finally eluted. The purified, high quality DNA is ready-to-use for subsequent downstream applications, e.g. for PCR amplification, quantitative PCR, real-time PCR or other routine downstream methods.

This manual contains 6 protocols.

Sampling and storage of starting material

Bacterial cultures
Bacterial cultures should be grown in presence of a selective agent such as an antibiotic. The yield and quality of DNA may depend on factors such as host strain, inoculation, antibiotic, and type of culture medium. The bacteria are centrifuged after cultivation. Best results are obtained with fresh or deep-frozen (-20°C or -80°C) material. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA.

Paper points
Paper points can be stored at room temperature for up to 6 hours. For long-term storage, we recommend a dry storage at 2-8°C.

Blood
Blood samples can be stored at room temperature for up to 6 hours or at 2-8°C for up to 24 hours. For long-term storage, we recommend freezing and storing samples at -20°C or -80°C. Multiple thawing and freezing cycles before isolating the DNA should be avoided. If cryoprecipitates (formed during thawing of frozen samples) are visible, avoid aspirating them. The amount of purified DNA from up to 100 µl whole blood depends on the white blood cell content. Different primary tubes and anticoagulants (except heparin) can be used to collect blood samples.

Tissue/ biopsy material
Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA. Use of poor quality starting material also leads to reduced yields. The amount of purified DNA from up to 10 mg tissue sample depends on the nature of starting material.

Urine
The bacteria must be centrifuged and the supernatant is completely removed (urea contaminations can inhibit PCR reactions). Best results are obtained with fresh or deep-frozen bacterial pellets. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA. The amount of purified DNA from up to 15-50 ml urine depends on the bacterial titer.

Swabs, saliva
The protocol works with fresh saliva, prepared swabs as well as with dried swabs. Please note that stored and dried swab samples are often characterized by isolation of apoptotic DNA (visible on agarose gel as a typical apoptotic DNA pattern).The protocol has not been validated for isolation of DNA from swabs which are stored in special storage buffers from other providers.

Pathogens (Listeria ssp.) in food material
For the detection of bacteria (Listeria) in foods an enrichment and cultivation, following the EU regulations and §35 of the food law, has to be done. An aliquot of the prepared culture is used and the bacteria are centrifuged after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this may lead to degraded DNA.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Procedure

Normalization
Each sample is mixed with 1x PBS or dd-H_2O and filled up to a final volume of 100 µl.

Sample preparation

Bacteria must be cultivated at special conditions. An aliquot of the suspension is used to form a bacterial pellet by centrifugation at high speed for 5 min while the supernatant is removed.

Lysis
Lysis is performed in two steps. First the cell wall of the bacteria is destroyed in the presence of **Lysozyme.** Then the samples are lysed at denaturing conditions and elevated temperatures while continuously shacking using **Lysis Buffer HL** and **Proteinase K**. DNases are inactivated whereas the bacterial DNA is secured.

Binding genomic DNA
Upon adding **Binding Buffer HL** and **MAP Solution A** to the lysate, optimal binding conditions are adjusted. The DNA is bound to the magnetic beads which will be mixed and transported by the magnetic rods.

Removing residual contaminants
Contaminants are efficiently removed using different **Wash Buffers** while the bacterial DNA remains bound to the beads.

Elution of pure genomic DNA

The bacterial DNA is finally eluted from the beads using **Elution Buffer**. The eluted DNA is ready-to-use in different downstream applications.

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the kit and its components as well as the package for any apparent visible damages and correct quantities. If there are any unconformities, notify STRATEC Molecular immediately in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, because their use may lead to poor kit performance.

- Always change pipet tips between different liquid transfers. To avoid cross-contaminations, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Only combine components of different kits if the lot numbers are identical.
- Avoid microbial contaminations of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend preparing the samples using a laminar air-flow box.
- The kit should only be used by trained personnel.

Important indications

- Optimal disruption of tissue is important for obtaining maximum yields and purities. For high sample throughput it is recommended to use a mixer mill or a similar device for disruption.
- It is recommended to centrifuge the isolated DNA for 1 min at maximum speed before starting any application.
- It is recommended to use multi-channel pipettes, esp. electric multi-channel pipettes with a capacity of 1 ml.
- Bacteria must be cultivated at special conditions. An aliquot of the bacterial suspension is used to obtain a pellet by centrifugation at high speed for 5 min. The supernatant is discarded.
- See sample preparation (page 12)

Preparing reagents and buffers

Prior to each run

Before starting a run, equilibrate all reagents at room temperature. Where necessary, gently mix and redissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

Lysis Buffer HL and **Elution Buffer**: are ready-to-use.

Lysozyme: Lysozyme should be prepared directly before starting a run. Solve the lyophilized powder by adding Lysozyme Buffer. Mix thoroughly until the entire enzyme is solved. For long-time storage we recommend to prepare aliquots and store them at -20°C. Avoid repeated freezing and thawing cycles.

Proteinase K: Add the described amount of ddH_2O (see table below) to the tube or bottle containing the Proteinase K, mix thoroughly and store unused Proteinase K at -20°C. Dividing the solved Proteinase K into aliquots for long-term storage is recommended. Avoid repeated freezing and thawing cycles.

Wash Buffers: Before first use, add the described volume of 96-100% ethanol to the bottles labeled with Wash Buffer I and Wash Buffer II as described in the Kit content (page 2) or see below. After addition of ethanol mix shortly and keep unused bottles firmly closed!

Binding Buffer: Add the described volume of isopropanol to the bottle labeled with Binding Buffer HL as described in the Kit content (page 2) or see below. After addition of isopropanol mix shortly and keep unused bottles firmly closed!

Preparing buffers

1x 96 bacterial DNA-extractions
Add 24 ml of 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking for 1 min. Mix thoroughly before each use. Dilute each **Proteinase K** tube by addition of 1.1 ml of ddH_2O, mix thoroughly until completely dissolving and store at -20°C! Dilute all **Lysozyme** directly before use with all of the provided **Lysozyme Buffer**. Store diluted Lysozyme at -20°C Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II,** mix thoroughly and always keep the bottle firmly closed
5x 96 bacterial DNA-extractions
Add 90 ml of 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Mix thoroughly before use Dilute **Proteinase K** by addition of 10 ml of ddH_2O, mix thoroughly until completely dissolving and store at -20°C! Dilute all **Lysozyme** directly before use with all of the provided **Lysozyme Buffer**. Store diluted Lysozyme at -20°C Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Vortexer
- 96-100% ethanol
- Isopropanol*

*The **InviMag® Universal Bacteria Kit /KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Scheme of the InviMag® Universal Bacteria Kit /KF96

Each sample with a volume smaller than 100 µl must be adjusted with dd H_2O or 1x PBS to a final volume of 100 µ. During lysis, prefill all Deep Well Plates with the required buffers and appropriate volumes as shown below:

Tip Plate: Insert the KF96 Tip Comb for DW magnets on a Tip Plate*

Binding Plate: Add to 470 µl lysed sample (total), 250 µl **Binding Buffer HL** and 20 µl **MAP Solution A** into a 2 ml Deep Well Plate

Washing Plate 1: Add 500 µl **Wash Buffer I** into a 2 ml Deep Well Plate

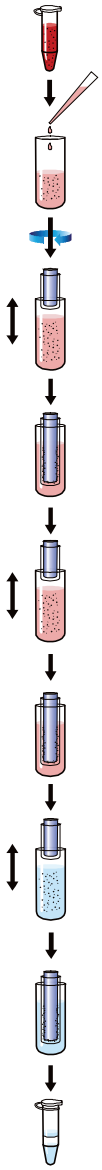
Washing Plate 2: Add 500 µl **Wash Buffer I** into a 2 ml Deep Well Plate

Washing Plate 3: Add 800 µl **Wash Buffer II** into a 2 ml Deep Well Plate

Washing Plate 4: Add 800 µl **Wash Buffer II** into a 2 ml Deep Well Plate

Elution Plate: Add 200 µl **Elution Buffer** into the KF Elution Plate (same size as Tip Plate)

Attention: Tissue and food samples require a modified protocol!



The prepared starting material is lysed in presence of **Proteinase K**, **Lysozyme** and **Lysis Buffer HL**

After lysis, a pause occurs and **Binding Buffer HL** *(follow preparing instructions)* and **MAP Solution A** (magnetic beads) are added to each sample containing cavity

DNA binds to magnetic beads

Magnetic separation

Four washing steps of the particle fixed DNA using **Wash Buffer I** and **Wash Buffer II**

Magnetic separation & evaporation of ethanol

Elution of DNA using **Elution Buffer**

Bead removal

Pure DNA

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.

Sample preparation

Bacteria must be cultivated at special conditions. An aliquot of the suspension will be used to form a bacterial pellet by centrifugation at high speed for 5 min while the supernatant is discarded.

General considerations

A summary of sample setup is described in the table below:

Sample and sample size	Condition	Treatment
10-100 µl blood	Fresh / frozen with anticoagulants	Adjust to 100 µl with ddH_2O/PBS
10-100 µl serum, plasma	Fresh / frozen	Adjust to 100 µl with ddH_2O/PBS
15-50 ml urine	Fresh / frozen	Centrifuge the sample and resuspend the pellet in 100 µl ddH_2O/PBS
5–10 mg tissue sample	Fresh / frozen	Completely homogenize the sample by crushing (mortar and pestle) or use a mixer mill for disruption. Resuspend the crushed sample with 100 µl ddH_2O/PBS
5–10 mg biopsy	Fresh / frozen	
5–10 mg frozen section	Fresh / frozen	
swabs	Fresh / dried	Adjust to 100 µl with buffer (0,4% SDS, 50 mM Tris-Hall; pH 8.0); or use 100 µl of transport medium or rinsed liquid
10–100 µl bacterial culture	Fresh / frozen	Adjust to 100 µl with ddH_2O/PBS
cell pellet from up to $1x10^9$ bacteria	Fresh / frozen	Resuspend in 100 µl ddH_2O/PBS

Starting a run - Protocol for isolation of bacterial DNA from clinical samples, bacterial cultures, swabs or urine

Before starting the purification process with the KF96 / KFflex96 please carefully read the manufacturer´s manual.

Prepare the plates of the instrument with the following buffers and follow the lysis and extraction process described below. Avoid evaporation of prefilled buffer components.

Attention: Please be aware, that Binding Buffer HL has to be prepared (see page: 13)

Note: Vortex the magnetic beads (MAP Solution A) thoroughly before each use!

Binding Plate: Add 100 µl **Lysozyme Buffer with Lysozyme** and 100 µl **sample** to a 2 ml Deep Well Plate

Washing Plate 1: Add 500 µl **Wash Buffer I** to a 2 ml Deep Well Plate

Washing Plate 2: Add 500 µl **Wash Buffer I** to a 2 ml Deep Well Plate

Washing Plate 3: Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate

Washing Plate 4: Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate

Elution Plate: Add 150 µl **Elution Buffer** into the KF Elution Plate

Tip Plate: Insert a KF96 / KFflex96Tip Comb for DW magnets on KF96 Plate

- Choose the program "**InviMag Uni Bacteria KF96**" or "**InviMag Uni Bacteria KFflex96**" depending on the used instrument
- Start the assay file by pressing the "Start" button of the instrument
- Load the plates into the machine as shown on the King Fisher display and confirm every plate loading step with the "Start" button

The following steps will run on the KF96 / KFflex96 instruments with only small user interaction:

1. Bacterial lysis

Transfer the whole sample (about 100 µl) to the Binding Plate and add 100 µl Lysozyme Buffer with added Lysozyme to each sample containing well. After starting the run, a heating step of 10 min at 37°C is performed.

2. Program pause for adding Proteinase K

After the first heating step, a pause will occur and 20 µl Proteinase K has to be added to each sample containing cavity of the Binding Plate. After adding the reagent, reinsert the plate (watch the correct orientation) and press the "Start" button to continue with the program.

3. Program pause for adding Lysis Solution HL

After 10 min of heating, a second pause will occur and 200 µl Lysis Buffer HL has to be added to each sample containing cavity. After adding the reagent, reinsert the plate (watch the correct orientation) and press the "Start" button to continue with the program.

4. Program pause for adding MAP Solution A and Binding Buffer HL (follow preparing instructions)

After 5 min of further incubation a third pause will occur. At this step, 20 µl MAP Solution A and 220 µl Binding Buffer HL have to be added to each sample. After adding the reagent, reinsert the plate (watch the correct orientation) and press the "Start" button to continue with the program. After this step the instrument will continue without any further user interaction.

5. Binding of the DNA

Automatically sample mixing for 5 min. Bead separation. Moving of beads to Washing Plate 1.

6. First Washing

Automatically sample mixing for 1 min. Bead separation. Moving of beads to Washing Plate 2.

7. Second Washing

Automatically sample mixing for 1 min. Bead separation. Moving of beads to Washing Plate 3.

8. Third Washing

Automatically sample mixing for 1 min. Bead separation. Moving of beads to Washing Plate 4.

9. Fourth Washing

Automatically sample mixing for 1 min. Bead separation.

10. Drying

Drying of beads outside of Washing Plate 4 for 5 min. Moving of beads to Elution Plate.

11. Elution of the DNA

Incubation of the beads into Elution Plate for 7 min. Bead separation.
The beads will automatically be discarded into Washing Plate 4).

At the end of the assay file the Elution Plate contains the extracted DNA which is ready-to-use in different downstream applications. Store the DNA at adequate conditions. We recommend storing the DNA at -20°C for long-term storage.
If the extracted DNA contains carryover of magnetic particles, transfer the eluate into a 1.5 ml reaction tube and centrifuge at maximum speed for 1 min. Transfer the supernatant to a new tube.

Starting a run - Protocol for isolation of bacterial DNA from tissue samples

Before starting the purification process with the KF96 / KFflex 96 please carefully read the instrument manual.

Prefill the plates of the instrument with the following buffers, respectively, and follow the lysis and extraction process described below. Please avoid evaporation of prefilled buffer components.

Attention: *Please be aware, that Binding Buffer HL has to be prepared (see page: 13*

Note: Vortex the magnetic beads (MAP Solution A) thoroughly before each use!

Tip Plate: Insert KF96 / KFflex 96Tip Comb for DW magnets on KF96 Plate

Binding Plate: Add 5-10 mg of **disrupted tissue**, resuspended in 100 µl **water or PBS** to a 2 ml Deep Well Plate and add 20 µl **Proteinase K**.

Washing Plate 1: Add 500 µl **Wash Buffer I** to a 2 ml Deep Well Plate

Washing Plate 2: Add 500 µl **Wash Buffer I** to a 2 ml Deep Well Plate

Washing Plate 3: Add 800 µl **Wash Buffer** II to a 2 ml Deep Well Plate

Washing Plate 4: Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate

Elution Plate: Add 150 µl **Elution Buffer** to a 2 ml Deep Well Plate

- Choose the program **"InviMag Uni Bacteria KF96 Cell"** or **"InviMag Uni Bacteria KFflex96 Cell"** depending on the used instrument
- Start the assay by pressing the "Start" button of the instrument
- Load the plates into the machine as shown on the King Fisher display and confirm every loading step by pressing the "Start" button.

The following steps will run on the KF96 / KFflex96 system with only minor user interaction:

1. Tissue lysis

Tissue disruption can be achieved by two main approaches: One way is to disrupt the tissue material by using a mortar and pestle in combination with liquid nitrogen. The homogenate is then resuspended in 100µl distilled water or PBS and transferred to the Binding Plate. Another approach is to directly add 100 µl distilled water or PBS to the tissue and the use a mixer mill for disruption. After that, the homogenate is transferred into the Binding Plate.

2. Bacterial lysis / Program pause for adding Lysozyme

After starting the run, a first incubation step of 30 min at 37°C is performed. After this lysis step, a pause occurs and 100 µl **Lysozyme Buffer** with added **Lysozyme** has to be added to each sample containing well. The run continues with a heating step of 10 minutes at 37°C.

3. Program pause for adding Lysis Solution HL

After lysozyme treatment another pause occurs. Now 200 µl **Lysis Buffer HL** has to be added to each sample containing cavity. Press the "Start" button again to continue with the protocol.

4. Program pause for adding MAP Solution A and Binding Solution HL *(follow preparing instructions)*

After 5 minutes of further incubation a third pause will occur. At this step the user has to add 20 µl **MAP Solution A** and 220 µl **Binding Buffer HL** to the lysed sample. From here the instrument will continue without any further user interaction.

5. Binding of the DNA

Automatically sample mixing for 5 min. Bead separation. Moving of beads to Washing Plate 1.

6. First Washing

Automatically sample mixing for 1 min. Bead separation. Moving of beads to Washing Plate 2.

7. Second Washing

Automatically sample mixing for 1 min. Bead separation. Moving of beads to Washing Plate 3.

8. Third Washing

Automatically sample mixing for 1 min. Bead separation. Moving of beads to Washing Plate 4.

9. Fourth Washing

Automatically sample mixing for 1 min. Bead separation.

10. Drying

Drying of the beads outside of Washing Plate 4 for 5 min. Moving the beads to Elution Plate.

11. Elution of the DNA

Incubation of the beads into Elution Plate for 7 min and bead separation. The beads will automatically be discarded into Washing Plate 4 (disposal).
After finishing the protocol, the Elution Plate contains the extracted DNA. Store the DNA under adequate conditions. We recommend freezing the DNA at -20°C for long-term storage.
If the extracted DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube and centrifuge at maximum speed for 1 min. Transfer the DNA into a new tube. The eluted DNA is ready-to-use in different downstream applications.

Protocol 1: Purification of bacterial DNA from clinical samples (serum, plasma, blood and cell free body fluid sample)

Please read the protocol prior the start of the preparation and complete preparing steps!

Use up to 100 µl whole blood (EDTA, citrate, *not heparin*), plasma or other cell free body fluid. For sample volumes smaller than 100 µl please use 1x PBS or distilled water to fill it up to 100 µl before starting the purification procedure.

1. Transfer the adjusted sample (100 µl) into a free cavity of the Binding Plate and add 100 µl Lysozyme, provided in Lysozyme Buffer.
2. Continue with the protocol as described in “Starting a run” at page 17.

Protocol 2: Purification of bacterial DNA from bacterial cultures

Please read the protocol prior the start of the preparation and complete preparing steps!

For isolation of DNA from bacterial pellets (up to $1x10^9$ bacteria) use an aliquot of the bacterial culture and centrifuge at 10.000 rpm for 3 min.

1. Carefully remove the supernatant without disturbing the pellet
2. Resuspend the pellet in 100 µl water or 1x PBS by pipetting up and down several times
3. Transfer the resuspended sample into a free cavity of the Binding Plate and add 100 µl Lysozyme, provided in Lysozyme Buffer.
4. Continue with the protocol as described in “Starting a run” at page 17.

Protocol 3: Purification of bacterial DNA from food samples

Please read the protocol prior the start of the preparation and complete preparing steps!

§ 35 LMBG (Law of food and utensil):

1. Weight in about 25 g of the food material and homogenize it.
2. Add 225 ml of the recommended culture media (e.g. Fraser media) to the homogenized material and cultivate it overnight (recommended time is 24 h).
3. Use a 1 ml aliquot of this culture and centrifuge it at 10000 rpm for 3 min.
4. Carefully remove the supernatant without disturbing the pellet
5. Resuspend the pellet in 100 µl water or 1x PBS by pipetting up and down several times
6. Transfer the resuspended sample into a free cavity of the Binding Plat and add 100 µl Lysozyme, provided in Lysozyme Buffer.
7. Continue with the protocol as described in “Starting a run” at page 17.

Protocol 4: Purification of bacterial DNA from swabs

Please read the protocol prior the start of the preparation and complete preparing steps!

It is recommended to rinse each swab with 500 µl PBS or distilled water. Use an aliquot of the rinsed liquid (100 µl) for the extraction of the bacterial DNA.
If the swab is delivered in a stabilization* media instead, use 100 µl of this medium.

1. Transfer 100 µl of the rinsed liquid or of the transportation media into the well of the Binding Plate and add 100 µl Lysozyme, provided in Lysozyme Buffer
2. Continue with the protocol as described in “Starting a run” at page 17.

(*) please contact STRATEC Molecular and ask for compatibility

Protocol 5: Purification of bacterial DNA from urine

Please read the protocol prior the start of the preparation and complete preparing steps!

1. Centrifuge the collected urine sample (15–50 ml) for 15 minutes at 1500 rpm.
2. Decant the supernatant carefully and resuspend the sediment in 3 ml PBS.
3. Centrifuge for 5 minutes at 3500 rpm.
4. Decant the supernatant completely by inverting the tube but do not disturb the pellet. It is very important to remove the supernatant completely! Residual amounts of liquids may have a negative influence on the extraction procedure!
5. Resuspend the pellet by addition 100 µl water or PBS by pipetting up and down several times.
6. Transfer the resuspended sample into a free cavity of the Binding Plate and add 100 µl Lysozyme, provided in Lysozyme Buffer.
7. Continue with the protocol as described in “Starting a run” at page 17.

Protocol 6: Purification of bacterial DNA from small tissue samples

Please read the protocol prior the start of the preparation and complete preparing steps!

1. Homogenize the 5–10 mg tissue samples using a Mixer Mill or liquid nitrogen in combination with a mortar and pestle.
2. Resuspend the crushed material in 100 µl water or PBS by pipetting up and down several times.
3. Transfer the resuspended sample into a free cavity of the Binding Plate and add 20 µl Proteinase K
4. Continue with the protocol as described in “Starting a run” at page 19.

For self-programming of the KF96 and KFflex96 instrument

Reagent info

Tip PLate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Binding Plate		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Sample	100	-	Sample
Lysozyme in Lysozyme Buffer	100	-	Reagent

Washing Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	500	-	Reagent

Washing Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	500	-	Reagent

Washing Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Washing Plate 4		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Elution Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	150	-	Reagent

Dispensed reagents

Binding Plate		Microtiter DW 96 plate	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Proteinase K	Add Proteinase K	20	-
Lysis Buffer HL	Add Lysis Buffer	200	-
Binding Buffer HL	Add Binding	220	-
MAP Solution A	Add Binding	20	-

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip PLate	
	Lysis1	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	No
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	37
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Add Proteinase K	Binding Plate	
		Message	Add Proteinase K
		Dispensing volume [µl]	20
	Reagent(s)	Name	Proteinase K
		Volume [µl]	20
	Lysis2	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Add Lysis Buffer	Binding Plate	
		Message	Add Lysis Buffer HL
		Dispensing volume [µl]	200
	Reagent(s)	Name	Lysis Buffer HL
		Volume [µl]	200
	Lysis3	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No

	Add Binding	Binding Plate	
	Reagent(s)	Message	Add Binding Buffer HL and MAP Solution A
		Dispensing volume [µl]	240
		Name	Binding Buffer HL
		Volume [µl]	220
		Name	MAP Solution A
		Volume [µl]	20
	Binding	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash4	Washing Plate 4	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5

	Drying	Washing Plate 4	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:07:00, Slow
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	5
	Bead Removal	Washing Plate 4	
		Release time, speed	00:00:30, Fast
	Leave	Tip PLate	

For self-programming of the KF96 and KFflex96 instrument (Cell protocol)

Reagent info

Tip PLate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Binding Plate		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Sample in distilled water/PBS	100	-	Sample
Proteinase K	20	-	Reagent

Washing Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	500	-	Reagent

Washing Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	500	-	Reagent

Washing Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Washing Plate 4		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Elution Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer D	150	-	Reagent

Dispensed reagents

Binding Plate		Microtiter DW 96 plate	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Lysozyme in Lysozyme Buffer	Add Lysozym	100	-
Lysis Buffer HL	Add Lysis Buffer	200	-
Binding Buffer HL	Add Binding	220	-
MAP Solution A	Add Binding	20	-

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip PLate	
	Lysis1	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	No
	Mixing / heating:	Mixing time, speed	00:30:00, Medium
		Heating temperature [°C]	37
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Add Lysozym	Binding Plate	
		Message	Add Lysozyme
		Dispensing volume [µl]	100
	Reagent(s)	Name	Lysozyme in Lysozyme Buffer
		Volume [µl]	100
	Lysis2	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	No
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	37
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Add Lysis Buffer	Binding Plate	
		Message	Add Lysis Buffer HL
		Dispensing volume [µl]	200
	Reagent(s)	Name	Lysis Buffer HL
		Volume [µl]	200
	Lysis3	Binding Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating temperature [°C]	60
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No

	Add Binding	Binding Plate	
		Message	Add Binding Buffer HL and Beads
	Reagent(s)	Dispensing volume [µl]	240
		Name	Binding Buffer HL
		Volume [µl]	220
		Name	MAP Solution A
		Volume [µl]	20
	Binding	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash4	Washing Plate 4	
	Beginning of step	Precollect	No
		Release time, speed	00:00:15, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5

	Drying	Washing Plate 4	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:07:00, Slow
		Heating temperature [°C]	50
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	5
	Bead Removal	Washing Plate 4	
		Release time, speed	00:00:30, Fast
	Leave	Tip PLate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA	insufficient lysis	increase lyses time, but prevent too long lyses time because this also decrease yield reduce amount of starting material
	incomplete elution	Use a higher volume of **Elution Buffer.** Ensure that the **Elution Buffer** is pipetted into the correct plate
	low amount of MAP Solution A	Mix **MAP Solution A** thoroughly before use
low concentration of extracted DNA	too much Elution Buffer	elute the DNA with lower volume of **Elution Buffer**
	incorrect storage of starting material	ensure that the storage of starting material is valid avoid multiple freezing and thawing cycles of the material
	incorrect Wash Buffers	ensure that the correct amount of ethanol was added to the **Wash Buffers** and storage conditions were kept as indicated
degraded DNA	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid multiple freezing and thawing cycles of the material
	old material	ensure that the starting material is fresh or stored at appropriate condition (for long-term storage at -80°C) avoid multiple freezing and thawing cycles of the material
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	ethanol carryover during elution	increase drying time for ethanol evaporation
	salt carryover during elution	check the **Wash Buffers** for salt precipitates. If any precipitates are visible, solve them by carefully warming up to 50°C ensure that the **Wash Buffers** are equilibrated at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA is brownish colored	small part of the magnetic particles are left in the elution	centrifuge at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

Note: *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA

The length and sensitive physical nature of DNA requires careful handling to avoid damage due to shearing or enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting or long-template PCR.

Storage of DNA

A working stock of DNA can be stored at 2–4˚C for several weeks. For long term storage DNA should be stored at -20˚C. Avoid repeated freeze-thaw cycles of the DNA to prevent irreparable damage of the DNA.

Note: *Please remind that the elution buffer will affect the stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis whereas Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acidic hydrolysis.*

Drying, dissolving and pipetting DNA

Avoid overdrying of genomic DNA after ethanol precipitation. We recommend to it air dry the DNA rather than to use a vacuum. Although, vacuum drying can be applied with caution.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA yield

The amount of purified bacterial DNA from cell-free body fluids depends on the microorganism content, sample source, transport, storage and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **InviMag®** procedure.

Ordering information

Consumables

Cat.no	Description
5400500	KingFisher 96 Magnetic Particle Processor, 100-240 V, 50/60 Hz (includes
24073430	KingFisher 96 Head for Deep Well plate
97002514	KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box
97002524	KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box
97002534	KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box
97002540	KingFisher 96 KF plate (200ul) 48 plates / box
95040450	Microtiter deep well 96 plate, 50 plates/box

Kit information

Product	Cat. No.	Package Size
InviMag® Universal Bacteria Kit/ KF96	7433300100	1 x 96 preps
InviMag® Universal Bacteria Kit/ KF96	7433300100	5 x 96 preps
InviMag® Bacteria DNA Mini Kit/ KFmL	2433150100	15 preps
InviMag® Bacteria DNA Mini Kit/ KFmL	2433150200	75 preps
Invisorb® Universal Bacteria HTS 96 Kit/ C	7033300300	4 x 96 preps
Invisorb® Universal Bacteria HTS 96 Kit/ C	7033300400	24 x 96 preps
Lysozyme	3020401200	500 mg
Lysozyme Buffer	3020401300	50 ml
RTP® Bacteria DNA Mini Kit	1033200200	50 preps
RTP® Bacteria DNA Mini Kit	1033200300	250 preps
RTP® Mycobacteria Kit	1033220200	50 preps
RTP® Mycobacteria Kit	1033220300	250 preps

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

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